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Agricultural Research Service

Vol. 35, No. 3, March 1987

# Agricultural Research



## SOIL WARS...

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Received by: MAY

Indexing Branch

## Viroid Discoverer Receives the Wolf Prize

1987 recipient of the \$100,000 Wolf prize in agriculture. The Israel-based Wolf Foundation annually awards prizes in agriculture, medicine, chemistry, physics, mathematics, and the arts. Chaim Herzog, president of Israel, will make this year's presentations at the Israeli Knesset in May.

Diener won the Wolf prize for discovering viroids in 1971 and his subsequent research on diseases that they cause.

Viroids are only the second disease-causing agent found in the 20th century. Discovery of the infectious particles, named viroids by Diener, is comparable to the discoveries of bacteria in the late 1800's and viruses in the first half of this century.

Although the name viroid implies they are like a virus, Diener says that is misleading. Viruses are composed of a core of nucleic acid (RNA or DNA) surrounded by a protective protein coat; viroids are simply fragments of RNA without a protective covering. They are about one-eightieth the size of the smallest known virus.

When a virus penetrates a cell, its nucleic acid takes over the role of directing cell activities and causes the cell to make more viruses like itself. This disrupts normal cell functions and thus causes disease.

The viroid also invades cells and disrupts their functions as effectively as any virus, despite its smaller size and lack of a protein coat.

Diener speculates that viroids may be very primitive viruses that have not developed the genetic sophistication to protect themselves with a protein coat.

Before the discovery of viroids, scientists believed that no viral nucleic acid with a molecular weight under 1 million hydrogen atoms could take over cell activities and reproduce itself, thus causing a disease. Diener's demon-

Theodor O. Diener, a plant pathologist with USDA's Agricultural Research Service, has been named the

stration of the infectious capabilities of viroids opened a new door for research into diseases which seem to be viral but whose causes have eluded scientists.

Sometimes called nature's littlest killers, viroids produce diseases in tomatoes, potatoes, citrus, and other crops. Chrysanthemum stunt disease, now known to be caused by viroids, almost wiped out chrysanthemums as a nursery crop in the 1950's.

Scientists speculate that viroids may be linked to several neurological diseases in animals and humans, including multiple sclerosis and infectious hepatitis.

Most of Diener's work has been on the viroid that causes a potato disease that makes the tubers spindle-shaped and reduces yield.

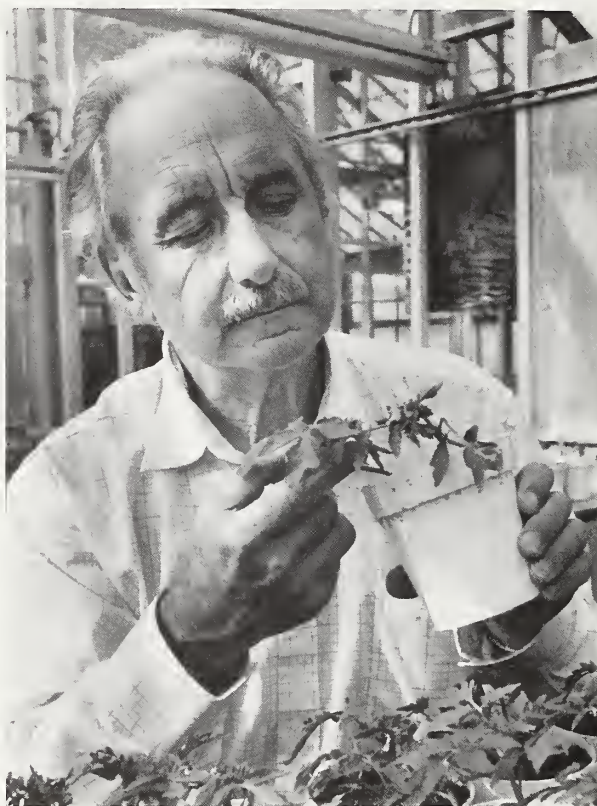
The disease had been known for at least 50 years before the discovery of viroids. It is easily spread by touching potato leaves with contaminated hands or tools and can be transmitted to successive generations through seed pieces, true potato seeds, and pollen. The disease becomes more severe as successive generations of infected potatoes are grown. In warmer climates, the loss is almost total unless viroid-free stock is planted.

Beginning his research in 1963 at the Beltsville (Maryland) Agricultural Research Center, Diener coaxed the viroids into revealing themselves by 1971.

In 1980, he and co-researchers Robert Owens and

Dean Cress made a mirror-image, complementary-DNA (cDNA) copy of the viroid in the laboratory. This copy was inserted into *E. coli* bacteria to be multiplied. The resulting viroid particles were used to develop a rapid method to check potato seed stock for the presence of potato spindle tuber disease. The test has been automated and included with other tests given to detect viruses in potatoes.

Diener is a member of the National Academy of Sciences, USA. He has won several awards over the past two decades for his research, including three from USDA, as well as the Campbell Award in 1968 and the Alexander von Humboldt Award in 1975.—L.E.M.



Theodor Diener examines tomato plants in his Beltsville (MD) greenhouse for symptoms of the viroid-caused potato spindle tuber disease. (1085X1204-15)

Tim McCabe

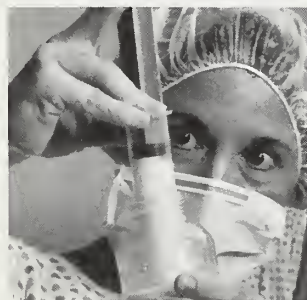


# Agricultural Research

Cover: Biocontrol fungi growing in petri dishes. After further growth in fermentation tanks, the fungi will be pelletized and field-tested against crop-damaging soilborne fungi. Story begins on page 6. Photo: Tim McCabe. (0187X039-14)



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Vol. 35, No. 3  
March 1987

Editor: Lloyd E. McLaughlin  
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and Anita Y. Daniels  
Art Director: Deborah Taylor

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*Agricultural Research* is published 10 times per year by the Agricultural Research Service (ARS), U.S. Department of Agriculture, Washington, DC 20250. The Secretary of Agriculture has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Send subscription orders to Superintendent of Documents, Government Printing Office, Washington, DC 20402. Information in this magazine is public property and may be reprinted without permission. Prints of photos are available to mass media; please order by month and photo number.

Magazine inquiries should be addressed to: The Editor, Information Staff, Room 318, Bldg. 005, Beltsville Agricultural Research Center-West, Beltsville, MD 20705. Telephone: (301) 344-3280. When writing to request address changes or deletions, please include a recent address label.

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## Predicting Wheat Development From Air Temperature

Using only air temperature records for their area, farmers can now predict how quickly their wheat will grow. These calculations help schedule critical farm operations.

"Many management decisions of small grain farmers—such as when to apply herbicides or fertilizer—require a knowledge of the stage of development of the wheat crop," says Ronald W. Rickman, a soil scientist at the Columbia Plateau Conservation Research Center in Pendleton, OR.

Rickman and his Agricultural Research Service colleagues in USDA have written the computations required for these temperature estimates into a computer program called PLANTEMP available from the Oregon State Cooperative Extension Service.

Poorly timed applications can damage the crop or reduce the effectiveness of weed or disease control. However, "With long-term average temperature records for a wheat-

growing area," says Rickman, "farmers can estimate future plant development and schedule management operations according to anticipated stages of wheat development."—By **Howard Sherman, ARS.**

*Ronald W. Rickman is at the USDA-ARS Columbia Plateau Conservation Research Center, P.O. Box 370, Pendleton, OR 97801. ■*

## Many Parts to Fresh-Squeezed Flavor

Orange juice has a flavor code that a USDA scientist in Winter haven, FL, has broken for the first time.

Manuel G. Moshonas, a chemist with the Agricultural Research Service, has identified 21 chemicals that contribute heavily to fresh orange juice's distinctive flavor. That natural blend of chemicals is altered when the juice is stored or processed.

"We found the 21 chemicals act as a flavor code that will help the citrus industry make processed juice

that tastes like it's just been squeezed from an orange," Moshonas says.

When processors make juice concentrate, they remove water and, inadvertently, some of the flavor chemicals. Later, water is added back along with some flavor ingredients, partially restoring the original taste.

Until now, scientists had not been able to break the flavor code because the chemicals are in low concentrations, and the water and sugars and other components make it hard for most instruments to measure the flavor ingredients.

Moshonas used low pressure and temperature to separate the water and flavor components from dissolved solids. Then he used a sensitive instrument called a gas chromatograph to separate and measure the components.

About 90 percent of the 200 million boxes of oranges produced in the United States each year are converted to processed products with a retail value of more than \$3 billion, according to figures from the Florida Department of Citrus.—By **Sean Adams, ARS.**

*Manuel G. Moshonas is at the USDA-ARS Citrus and Subtropical Products Laboratory, P.O. Box 1909, Winter Haven, FL 33880. ■*

## Soybean Roots Follow Wormholes

How far soybean roots travel seeking water and other nutrients may depend on the number of holes in the soil, such as those made by earthworms.

Doyle B. Peters, a soil scientist with the USDA Agricultural Research Service, and research associate Juan Wang, both at the University of Illinois in Urbana, say soybean roots that fail to enter burrows in the top 12 to 18 inches of soil often end up with dead root tips.

Once in an old worm or insect



Tim McCabe

A Midwestern farmer applies a pre-emergence herbicide to cropland. (IA-2876)

burrow, or a channel made by previous crop roots, soybean roots grow as far as the end of the channel—usually 4 to 5 feet.

The researchers, including ARS plant physiologists John D. Hesketh and Joseph T. Woolley, found 400 to 1,200 vertical burrows at 8-inch depths in each square yard of soil they sampled.

In general, the number of burrows increased with depth, down to 12 to 20 inches, then decreased. Few burrows were found below 52 inches.

“The number of burrows and the tendency of roots to grow in them,” says Peters, “suggests that burrows have a profound effect on the shape of the soybean root system as well as on the rapid air and water flow through the soil.”—By **Betty Solomon**, ARS (retired).

*Doyle B. Peters, John D. Hesketh, and Joseph T. Woolley are in USDA-ARS Soil, Water, and Plant Research, University of Illinois, 1102 South Goodwin Ave., Urbana, IL 61801. ■*

## It's a Mistake To Study Only Males

Vive la difference! is getting a new translation from nutrition studies which show it's the difference that keeps one sex long-lived.

According to biochemist Meira Fields, most studies are done on male animals and men to eliminate the variable of female hormone fluctuations. “We may be making a big mistake by generalizing from these studies,” she says, based on her research comparing both sexes.

Female rats survived copper-deficiency experiments that killed 40 to 70 percent of their male counterparts in just 8 weeks. After 5 weeks of eating the experimental diet, the male rats began dying from enlarged hearts that ruptured. Other studies have shown that female rats can survive a year on this diet, says Fields, who is based at the Agricultural

Research Service's Human Nutrition Research Center, Beltsville, MD. She is a research associate with Georgetown University Hospital, Washington, DC.

“It's a well known fact that premenopausal women are not susceptible to heart abnormalities,” she says. “Now we have the first experimental evidence that gender can protect against heart-related death.”

How this happens is unclear. Fields says that both sexes had similar low readings in the conventional tests used to assess body copper stores—indicating that these tests “do not accurately reflect the greater effect of deficiency in males.”

Both sexes also had elevated blood levels of cholesterol, triglycerides, and uric acid—symptoms known to increase risk of heart disease. But, she says, only the male rats were anemic and had enlarged hearts, signaling severe damage.

All these indicators of copper deficiency were provoked by feeding the animals high levels of fructose—a natural sugar abundant in fruit and increasingly refined for use in the U.S. food supply because it is twice as sweet as sucrose (table sugar).

“A copper-deficient diet is not enough,” says Fields. “You need fructose to aggravate it.”—By **Judy McBride**, ARS.

*Meira Fields is at the USDA-ARS Beltsville Human Nutrition Research Center, Room 312, Bldg. 307, Beltsville, MD 20705. ■*

## Building a Better Steak Burger

Consumers who can't afford a New York strip steak but are tired of eating ground beef may someday be able to select a steak made from cheaper cuts.

“As far as texture, restructured beef steaks are halfway between ground beef and a porterhouse, with less fat than hamburger,” says Steven C. Seideman, formerly a meat technologist with the Agricul-

tural Research Service and now director of research for Bryan Foods in West Point, MS.

Ground beef averages 20 to 30 percent fat. The restructured beef is 12 to 15 percent fat, he says. “It is not a whole muscle mass like a New York strip, but you can tell it's not ground beef,” says Seideman.

Some hotels, restaurants, and institutions, including the U.S. military, use restructured beef. Annually, 4 million pounds of restructured beef are eaten by military personnel in dining halls throughout the world.

Commercially, however, restructured beef fails to tickle the tastebuds of consumers. Researchers at the U.S. Meat Animal Research Center, Clay Center, NE, looked at the process of restructuring beef and made some improvements. “We wanted to get it to hold together better,” says Seideman.

The researchers added 1 percent salt (sodium chloride) to chunks of chuck meat and reshaped it. Adding salt helps the chunks hold together so the steaks must be cut with a knife, says Seideman.

When salt is added to meat, the amount of time the product can be frozen is reduced, says Seideman. Storage life for the restructured beef with added salt averages 3 to 4 months.

When the researchers added a half percent of a USDA- and FDA-approved food additive, sodium tripolyphosphate, to the restructured beef, storage life of the product increased to 5 months. The food additive also improved color and the way the beef held together.

Trained tasters evaluated the best steaks in the study as “moderately” desirable in flavor and overall satisfaction. Adding slightly more of the tripolyphosphate should improve the sensory, visual, and binding properties of the restructured steaks, says Seideman.—By **Linda Cooke**, ARS.

*This research was done at the USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933. ■*

## 240 Biological Defense for Many U.S. Crops 4



Beneficial fungus *Gliocladium virens* coils around and will eventually destroy larger *Rhizoctonia solani*, a soilborne pathogen that attacks more than 200 plant species. Magnified about 3,000 times. Scanning electronmicrograph by William Gurtshaw. (PN-7230)

Delivery systems are at hand for what amounts to soil wars against many crop diseases, say USDA Agricultural Research Service scientists.

Borrowing from biological struggles in nature, new crop-protecting systems are based on good molds that attack disease-causing molds in topsoil.

Each year, some 50 groups of soil-borne fungi and a few types of bacteria and viruses keep U.S. crops from producing an additional \$4 billion worth of harvest, says plant pathologist George C. Papavizas of USDA's Agricultural Research Service.

He and colleagues at the Soilborne Diseases Laboratory in Beltsville, MD, are working on ways to halt these losses by turning beneficial molds against the disease-causing organisms.

They have developed ways to grow good molds in a fermentation system, then mix them with inert powders or convert them into sprays and pellets.

"When we add these products to field or greenhouse soil, they shield crop plants from attacks by harmful molds. Safe, nonpolluting disease control may last for several seasons in some cases," says Papavizas.

After nearly 20 years of soil ecology studies to find and enhance the activity of disease-fighting molds, Papavizas and coworkers adapted industrial fermentation techniques to grow molds in the laboratory.

This, and the discovery of new beneficial molds, has led to rapid progress in the past 5 years.

The molds are fermented on cheap food sources, such as molasses and brewers' yeast. Gelling agents commonly used in food processing are added to make pellets that when dried resemble some plant fertilizers. The pellets are uniform in size, biodegradable, and nontoxic, Papavizas says.

Many companies are interested in marketing the product, particularly pharmaceutical or other industries that routinely use large-scale fermentation vats.

Papavizas and other scientists at the ARS Soilborne Diseases Laboratory at Beltsville, MD, hope that research on biological systems will help lower financial and environmental costs of chemical controls of crop diseases.

Also, the soil is difficult to penetrate with pesticides.

"Topsoil is the source for most of the pathogens of over 250 different crops. Given the microjungle of soil life, it's smart to work toward pest control within the balances of nature rather than to spray pesticides," says soil scientist Jack Lewis.

But beneficial molds cannot yet replace pesticides in all cases. They can often be used as one part of an integrated pest management strategy that has to be worked out for each disease. In an integrated strategy, molds are used along with small amounts of fungicides, resistant crop varieties, cultivation practices, and disease forecasting.

Here are highlights of the research to date.

- In tests, two harmful molds that cause cotton seedling disease were controlled just as well by beneficial molds *Trichoderma* and *Gliocladium* as with fungicide treatments. Cotton seedling disease is a major problem for U.S. cotton farmers.

One application of the same two good molds reduced disease on potato plants 2 years running. No molds were added to test plots for the second year; those applied previously had stayed "on guard."

- Ultraviolet treatments changed a strain of *Trichoderma*, called T-1-R9, so that it can tolerate the fungicide benomyl. When T-1-R9 is used in soil for chrysanthemums, for example, the fungicide necessary to control other diseases on the plant leaves won't kill the resistant T-1-R9 if it drips onto the soil. Used alone, T-1-R9 also lowered disease damage on greenhouse crops of chrysanthemums and carnations by 50 to 60 percent and on potatoes by 10 to 20 percent. The T-1-R9 mold is patented. (See page 16.)

- A new beneficial soil mold, *Sporidesmium sclerotivorum*, was discovered several years ago by pathologist Peter B. Adams of the Beltsville laboratory. Adams and colleague W.A. Ayers found that *S. sclerotivorum* destroys two important plant pathogens that cause severe economic losses to many vegetable, oilseed, and forage crops. They demonstrated that a single application of the beneficial mold to soil reduced lettuce disease by 65 to 85 percent. A patent was issued on a method of growing and using the beneficial mold for biological disease control. Industrial firms are now ex-

ploring ways to mass-produce the mold for agricultural use. One firm has succeeded in producing it in quantity. The good mold persists for years as it invades and destroys dormant forms of harmful fungi.

- Following successful tests of a pelletized mold, *Talaromyces flavus*, in 1984, soil populations of this beneficial mold increased tenfold in 2 years, says plant pathologist Deborah R. Fravel. In the second year, with no additional *T. flavus* applied, wilt disease in a test plot of potatoes was reduced 14 percent.

*T. flavus* added to potting soil for eggplants (a crop often started in greenhouses) before transplanting outdoors resulted in a 75-percent reduction in *Verticillium* wilt disease in the field.

- *T. flavus* is normally sensitive to fungicides that may be applied to control disease-causing molds. However, genes that help slow-growing strains of *T. flavus* resist fungicides were transferred in the laboratory to fast-growing strains by sexual crosses to upgrade the biocontrol efficiency of the mold.

- Disease-causing molds destroy up to 20 percent of U.S. bean and pea crops yearly. By themselves, fungicides are inadequate or too expensive, and resistant bean and pea varieties are largely ineffective, but a combination of *Trichoderma* powder, tolerant bean or pea varieties, and some chemical fungicides gave good control. Bean yields jumped 60 percent and peas 50 percent with the integrated control strategy.

- Two soilborne molds cause 10 percent disease losses in bedding plant crops (plants started in greenhouses for flower beds). In tests by plant pathologist Robert D. Lumsden, *Gliocladium virens*, a beneficial soil mold, added to potting mixes as fresh culture or in granular form controlled both harmful molds.

- Tests of pellet delivery systems for *Trichoderma* and *Gliocladium* showed a 75- to 95-percent reduction of *Rhizoctonia solani*, a fungal pathogen of roughly 200 economically important crops.—By **Stephen Berberich**, ARS.

*The scientists mentioned in this article except W.A. Ayers (retired) are at the USDA-ARS Soilborne Diseases Laboratory, Bldg. 011A, BARC-West, Beltsville, MD 20705. ■*



Soil scientist Jack Lewis with flats of zinnia seedlings that were exposed to the harmful mold *Pythium*. Healthier plants in tray on right were treated with the beneficial mold *Gliocladium* before exposure. (1186X1318-20)



Biodegradable, nontoxic, and inexpensive pellets contain a biocontrol fungus and its food base with a binding agent. Applied to soil like fertilizer, they shield crops from harmful fungi. (1186X1316-18)

## 245 A Little Stress Can Help Plants Cope //

In late March, a truck pulls up alongside a sunlit greenhouse in Florida. The driver loads flats of multi-colored coleus seedlings destined for garden centers in the Northeast. At the same time, a second driver loads flats of the same variety of the popular houseplant grown at the same temperature in a section of Florida that has been under dense clouds for 2 days. As both drivers head north through Georgia, the temperature drops to around 40°F. When the first driver reaches market the next day, the coleus leaves have begun to yellow from chilling damage. But the second driver arrives with perky plants that show no signs of the cold. Why?

According to USDA Agricultural Research Service plant physiologist Donald T. Krizek, a plant's "living conditions" before stress often determine whether the plant will overcome or succumb to that stress. In this hypothetical scenario, he explains, the coleus that were under low-light conditions for a few days before chilling were better prepared to handle the cold.

He and colleagues simulated these conditions in environmental growth chambers at the Beltsville (MD) Agricultural Research Center. They found that exposure to 2 days of low light levels under fluorescent lamps preconditioned coleus to withstand 40-degree temperatures; high levels of fluorescent light aggravated the plant's response to subsequent chilling, causing more damage than normal.

In this and other studies, the scientists are looking into how various stresses interact with one another: Can one stress protect a plant against a later stress or does it make the plant even more vulnerable? While a plant's genes play a big role in its tolerance of a given stress, the scientists are finding that preconditioning may be equally important. For example, their studies have shown:

- Subjecting coleus and poinsettia plants to moderately severe drought for about 2 days prepared them to survive in air heavily polluted with sulfur dioxide.

- Holding coleus and poinsettia at about 56°F for 5 days prepared them to withstand sulfur dioxide levels that would normally cause damage.

- Subjecting coleus to severe drought before exposing them to 2 days of 40-degree temperatures increased chilling injury in temperature-sensitive varieties and caused injury in normally cold-tolerant varieties.

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### ***Can one stress protect a plant against a later stress or does it make the plant even more vulnerable?***

Donald T. Krizek, ARS plant physiologist

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These findings should help explain why researchers around the world are getting different responses from the same plant varieties under the same stress conditions. "In the real world," Krizek says, "plants are subject to multiple stresses, but researchers generally study a plant's response to one stress at a time without taking into account the conditions that preceded the treatment."

On a more practical level, the findings indicate that growers can increase their profits by knowing what combinations of stresses help or harm their plants during production and shipping. Krizek and colleagues are developing general principles to help guide growers in applying the right stress at the right time. This will also help breeders in selecting the right combination of genes for broad tolerance to stresses.

"Part of stress tolerance is stress avoidance," explains Krizek. For instance, several kinds of stress regulate the opening and closing of the stomates—the pores in plant leaves through which gases and water vapor pass. "If the stomates close to conserve water during drought, this also blocks the entry of gases such as sulfur dioxide."

Preconditioning can help sensitive plants overcome their own genetic

shortcomings. Krizek and horticulturist Peter Semeniuk induced varieties most sensitive to sulfur dioxide pollution to become insensitive by preconditioning them with moderate drought or spraying well-watered plants with a plant hormone—abscisic acid—that causes the stomates to close.

Perhaps the most important findings, commercially speaking, are coming from current research into the interaction between pot size and lack of water. The containerized plant business is a booming industry.

Conventional wisdom holds that small pots restrict plant growth by limiting the volume of roots available to take up water—thereby simulating drought conditions. Cooperative studies with plant scientists in Israel, at the University of Maryland, and at other ARS locations dispute this notion. The studies represented three major plant categories—soybeans, tomatoes, and the woody shrub euonymus.

Small pots and drought each reduced overall growth by reducing the number of lateral branches the plants formed. "The fact that the plants weren't as bushy," says Krizek, "suggests an imbalance in cytokinins and other plant hormones." But the similarity ended here.

Small pots had no effect on the formation of new leaves or the rate of photosynthesis—the process by which plants manufacture their own food. Too little water, on the other hand, slowed both processes. When photosynthesis is slowed down, it limits the food supply needed for optimum growth, he explains. Drought also produced a build-up of carbohydrates in the plant tissue, indicating that the plants were less able to metabolize the food they produced.

"With frequent watering," Krizek says, "you can eliminate some of these problems in small pots."—By **Judy McBride**, ARS.

Donald T. Krizek is in the USDA-ARS Plant Stress Laboratory, Room 206, Bldg. 001, Beltsville Agricultural Research Center, Beltsville, MD 20705. ■

## Ticks Look Up When Hitching a Ride

When its view of blue sky or green plants is blocked by people or animals, a tick knows a potential host is nearby, report Lawrence G. Pickens and John F. Carroll of USDA's Agricultural Research Service.

Ticks, such as the American dog tick, don't actually see colors but distinguish shades of gray, according to the two entomologists who are based at the agency's Agricultural Research Center in Beltsville, MD. "It's like what you see when a color program is shown on a black and white TV," Pickens says.

These findings are from a 4-month laboratory study of two tick species. It is the first showing that ticks react to different wavelengths of light.

But blue or green clothing won't fool a tick, because color is only one clue a tick uses to find a host. A tick can sense vibrations and carbon dioxide, which tell it that something nearby is moving and breathing. Some ticks respond by sticking out their front legs to hitch a ride.

"Our findings may lead to control methods in the long term," Carroll says, "but for now they help us with a basic understanding of how ticks see and why they act the way they do."

Pickens and Carroll began with the camel tick, an Egyptian species provided by a quarantine research laboratory at Old Dominion University in Norfolk, VA. They chose the camel tick because it has relatively large eyes.

Using special electronics equipment and color filters, they found that the camel tick responded most strongly to blue light and either stopped running or changed direction when even weak blue light was blocked. That may be because the camel tick's eyes face upwards.

"If something above them blocks out the blue sky, it might be a camel, cow, horse, or other animal," Pickens says. "That's all the tick needs to know."

The other tick, the American dog tick, can carry Rocky Mountain spotted fever and is common in the eastern United States. This tick's hitchhiking



Bob Bjork

response to blocked green light may occur because its eyes are on the edge of its body, and it sits on green plants waiting for a host to come along.

To measure each tick's reactions to light, the scientists hooked up a tiny electrode—thinner than a piece of hair—to its optic nerve. This nerve carries electronic pulses from the eye to its brain.

The electrode picked up these pulses, which were boosted through an amplifier and displayed on a video screen. The larger the pulse on the screen, the stronger the tick's sensitivity to a particular wavelength of light.—By **Sean Adams**, ARS.

*John F. Carroll and Lawrence G. Pickens are at the USDA-ARS Livestock Insects Laboratory, Beltsville, MD 20705. ■*

An American dog tick, common in the eastern United States, is plucked from a person's arm. (1086X1204-22)

# Bioengineered Chickens for the Future



Lyman Crittenden (left) holds a rooster he injected with genes of avian leukosis virus when it was a 1-day-old embryo. Donald Salter (center) and Leonard Provencher hold roosters of two succeeding generations, which directly inherited these virus genes. Viruses may someday be used as carriers for genes for leaner, tastier, bigger, and more profitable chickens. (1286X1416-8a)

Some very special chickens make East Lansing, MI, their home these days.

They look like the hundreds of other White Leghorns at the Agricultural Research Service poultry laboratory. But their genes now contain a "locomotive" that two scientists—geneticist Lyman B. Crittenden and microbiologist Donald W. Salter—hope can carry poultry bioengineering into the future.

Strangely, this locomotive is made of the genes of a weak form of the avian leukosis virus, which can lower egg production and produce tumors in poultry.

The leukosis virus is what makes the East Lansing chickens so special—it's a retrovirus, which lacks DNA. All viruses trick host cells into making new virus particles, but retroviruses invade the cell's gene structure to do this.

"Once inside a cell, a retrovirus releases an enzyme that converts its RNA to DNA," Crittenden explains. "The DNA seeks out the cell's genes and merges with them, and the cell then produces a new virus particle."

"What we did was inject the retrovirus into hundreds of fertilized, day-old embryos which we then hatched in an incubator. If tests showed the DNA of a chicken's blood cells contained the virus genes, we bred that chicken with one free of the virus and tested their progeny to find ones that inherited the virus genes from the parent. Then we repeated that step for a second generation of chicks and found that the viral genes had become a stable part of the chick's genetic library."

Researchers from the National Institutes of Health's Cancer Research Facility in Frederick, MD, and from the Veterans Administration in Salt Lake City, UT, furnished technical assistance in preparing batches of the virus genes for insertion.

Crittenden and Salter foresee that after many more years of research, retrovirus genes could carry into chickens other genes that would enable chickens not only to resist the disease but also to lay bigger eggs, grow larger on less food, be less fatty, taste better, or have other traits producers look for.

Furthermore, the chickens would transmit those traits to progeny.

To use retroviruses in bioengineering research, scientists would first have to couple the virus genes—the locomotive—to the gene or genes of research interest, and then insert this genetic "freight train" into embryonic cells.

Crittenden and Salter believe that harmless genes of the virus could be perfected for this purpose within 5 to 10 years.

"With more research, turning the virus against itself may be the first practical achievement of exploiting its need to use a cell's genes to reproduce," says Crittenden.

"To do that," says Salter, "we would have to insert defective virus genes. These could not produce virus particles but could block similar disease-causing viruses from entering a cell."

Crittenden cautions that chickens and other farm animals could have custom-inserted genes to control growth and other functions only after scientists learn much more about which genes or gene groups regulate these functions.

Robert J. Wall is a member of an ARS team placing genes into farm animals at the Agricultural Research Center in Beltsville, MD. "Retroviruses," he says, "could give us a much more efficient way to integrate new genes in pigs, sheep, and cows."

The usual method of inserting genes, microinjection, is much more difficult. In microinjection, genes are injected into the pronucleus formed from a sperm cell after the sperm enters an egg but before its chromosomes merge with those of the egg.

Crittenden and Salter used a simple drill to open a hole in the eggshell, a syringe to squirt the virus into the yolk, and a bit of glue to patch the hole.

A 25-year ARS veteran, Crittenden was named one of the agency's Outstanding Scientists for 1985 for his work with poultry viruses.

The studies are reported in the March issue of the journal *Virology* and patent protection has been applied for.—By **Jim De Quattro**, ARS.

*Lyman B. Crittenden and Donald W. Salter are at the USDA-ARS Regional Poultry Research Laboratory, 3606 East Mount Hope Rd., East Lansing, MI 48823. ■*

Robert Barclay

# 246 The Sunshine Vitamin Isn't Just Kid Stuff

Vitamin D deficiency among the elderly, especially those who live in northern climates, may be a major unrecognized epidemic.

"They're not getting enough of the vitamin from their diets, especially milk, or from short periods of exposure to summer sunlight," according to Michael F. Holick, who heads the vitamin D and bone metabolism lab at the Human Nutrition Research Center on Aging. USDA's Agricultural Research Service funds the center based at Tufts University, Boston.

"Adding a vitamin D deficiency on top of osteoporosis may significantly increase the risk of bone fractures," he says.

**"The major cause of age-related vitamin D deficiency is a decrease in milk consumption."**

Michael F. Holick, head of the Vitamin D and Bone Metabolism Laboratory

In a study of 142 patients entering Boston's Massachusetts General Hospital with fractured hips, 30 to 40 percent had little or none of the circulating form of vitamin D—the best index of nutritional status—in their blood, says Holick. He collaborated on the study with Samuel H. Doppelt and Robert M. Neer of the hospital's Mineral Metabolism Unit.

Holick says that other studies in the United States and Great Britain indicate that a similar 30 to 40 percent of men and women with hip fractures are vitamin D deficient.

It is estimated that about 200,000 Americans suffer from hip fractures each year, costing some \$2 billion in medical treatment.

Vitamin D helps the body absorb calcium from the intestinal tract and maintains blood levels essential for proper mineralization of the bone, Holick explains. Deficiency in adults leads to softening of the bone, or osteomalacia, compounding the problem of bone loss (osteoporosis) in the elderly.

People begin losing bone mass during their thirties, leading to a weakened, spongelike bone structure in later years. Although osteoporosis is accelerated in postmenopausal women, "all people have it sooner or later," he says.

"The major cause of age-related vitamin D deficiency is a decrease in milk consumption," Holick says. "Relatively few foods contain vitamin D, and the principal food source is milk." He says a substantial number of elderly develop an intolerance to the milk sugar lactose, and others believe there is no need for milk—that it's only for children.

Four 8-ounce glasses of milk a day provide the Recommended Dietary Allowance of 400 international units of vitamin D, he says. Depending on the fat content, 32 ounces of milk also supply about 1,200 milligrams of calcium—or one-third more than the RDA for calcium for men and women over age 18.

People who can't tolerate milk should take a vitamin D supplement or spend more time in the summer sun, Holick says. As people age, the ability of sun to stimulate the manufacture of vitamin D in the skin progressively decreases. In the laboratory, he found that skin samples from 8 to 18 year olds made two to three times more of the vitamin than skin samples from 80 year olds.

But vitamin D synthesis stops completely in the winter in latitudes as far north as Boston and beyond. And one form of the vitamin—vitamin D<sub>3</sub>—is actually broken down and inactivated by winter sunlight.

To get the benefits of summer sunlight and minimize its cancer-causing



Tufts University researcher Ann Webb places human blood cells under a solar simulator to determine sunlight's role in vitamin D production in the body. (0286X133-11)

effects, Holick recommends light-skinned elderly people restrict exposure to 5 to 10 minutes around midday in June when the sun's rays are most direct. Exposure can be longer when the angle of the sun is lower.

Dark-skinned people, however, require five to ten times longer in the sun, depending on the amount of pigmentation, he says. In laboratory tests, it took a dose of radiation that would produce a severe sunburn in Caucasians to increase vitamin D levels in black skin.

Holick also found that protective lotions called sunblocks are true to their name. The products with a high protection index completely prevent the skin from producing vitamin D.—By Judy McBride, ARS.

Michael F. Holick is at the USDA-ARS Human Nutrition Research Center on Aging, 711 Washington St., Boston, MA 02111. ■

## 245 Infant Formula— More Like the Real Thing 4

*"Development, growth, and vital activity all depend upon the availability of food in proper amounts and proper quality," Russell H. Chittenden wrote in Nutrition of Man in 1907.*

"Today, the connection between diet and health is well established, although not fully understood," says Buford L. Nichols, scientific director of the Children's Nutrition Research Center in Houston, TX. The center is a facility of USDA's Agricultural Research Service located at Baylor College of Medicine and Texas Children's Hospital in Houston.

Good health obviously benefits the individual, but a healthy population is also important to the Nation's economic and social well-being, Nichols says. Since a great deal is known about the dietary needs of the general public, modern-day nutrition research is increasingly focused on the needs of specific groups.

To one such group under study at the Houston center—infants—mother's milk represents the best diet known, at least for most babies. Yet, for a variety of personal, economic, or social reasons, slightly over a third of American mothers quickly introduce their babies to the bottle.

It is for this third, some 1,400,000 newborn infants each year, that researchers are analyzing human milk to gain an understanding of its composition and how its dietary benefits may be incorporated into infant formulas.

Richard J. Schanler, a researcher who specializes in medical disorders of the newborn, has found that premature babies digest triolein, a form of dietary fat, more completely when they receive it in their own mother's milk instead of in standard infant formula.

Working with Schanler in the triolein studies, Peter Klein, leader of the center's Stable Isotope Program, says that differences in the oxidation of specific fats in human milk and infant formulas can be measured by attaching a nonradioactive stable isotope to the triolein.



Jack Dykinga

Judy Hopkinson prepares mother's milk for storage in the ARS Children's Nutrition Research Center milk bank, for later research. (0986X1108-19)

The isotope is an atom that can be distinguished from others by its weight. Klein says, "It functions as a label or tag, allowing us to safely track the movement and absorption of fat in the body. This technique also enables us to study the development of a baby's small intestine during the first few weeks after birth—with no stress to the child."

Present estimates of the amount of food infants need for growth are imprecise because they are based on mainly theoretical figures, says Corinne M. Montandon, the center's administrative nutritionist. Calorie intakes of formula- and breast-fed infants are about the same at 1 month of age, she says, but by 4 months, formula-fed babies consume significantly higher amounts than those being breast-fed.

The higher energy consumption by bottle-fed babies may mean that they have different patterns of growth than breast-fed babies. Nancy F. Butte, a nutrition scientist in the center's Lactation Program, found that breast-fed infants gain weight about as fast as formula-fed babies who consume more protein and energy.

"Despite their lower intakes," she says, "breast-fed infants just seem to use the protein and energy in mothers' milk more efficiently than other infants use the nutrients in formulas."

Cutberto Garza, leader of the Lactation Program, agrees. One reason for the better dietary efficiency is the fact that breast-fed babies have a relatively low rate of infections, he says.

Recent studies show that protective factors, such as antibodies to fight infections, are excreted in higher amounts in the wastes of breast-fed infants than in those of their bottle-fed counterparts. Previous researchers had concluded that breast-fed babies received these protective factors from their mother's milk.

"But this may not be the whole story," says Garza. "Human milk may include some unidentified stimulators of the infant's immune system. In other words, mothers' milk not only supplies some protective factors but also helps babies develop their own. Eventually

we hope the immune systems of bottle-fed babies can be stimulated in a similar way."

Garza continues, "Excessively high or low sodium (salt) in infant diets presents a health risk." He says diets with too much sodium may increase the risk of hypertension later in life for infants who are genetically predisposed to this disease. On the other hand, too low sodium levels can lead to more immediate health problems. Salt loss during diarrhea is a primary cause of serious illness in infants.

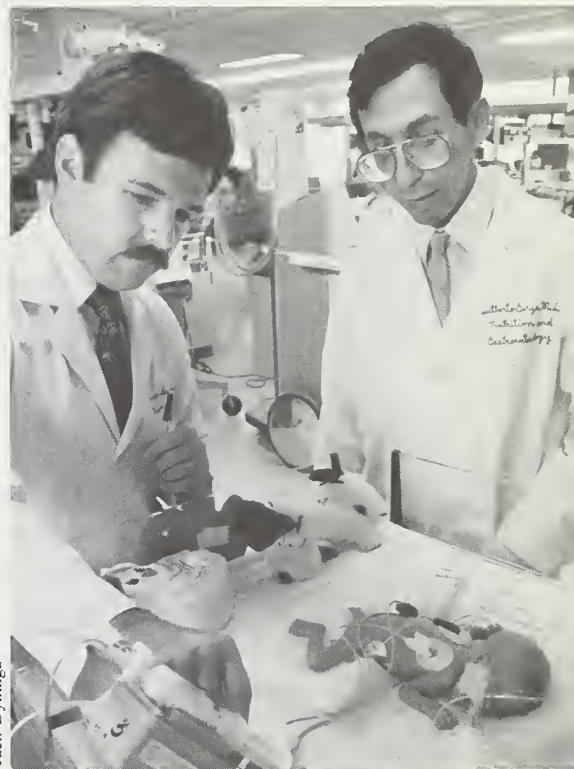
The milk from some mothers is quite low in sodium, Garza says, and not directly controlled by the mothers' diet. Recent results show that hormones exert the greatest influence on salt metabolism in infants and their mothers. "We do not know how infants adapt to these low intakes, nor do we understand why diarrheal illness in breast-fed infants is less severe than in those who are bottle-fed," Garza says. "But we hope to determine whether the same economy of sodium use can be achieved in formula-fed infants."

### Supplementing Infant Formulas To Restore Normal Growth

"About 1 in 20 American babies suffer from stunted growth in their formative years, generally as a result of chronic diseases, such as diarrhea," says Robert J. Shulman, a pediatrician at the center. "These diseases often reduce the quality or quantity of food nutrients absorbed by the body and, unless corrected, can stunt a child's growth forever."

In studies of the developing gastrointestinal tract, Shulman and coworkers found that even healthy babies have a limited capacity to digest glucose polymers, the subunits of complex carbohydrates such as starch. One-month-old babies digest short-chain polymers completely, but their absorption of long-chain polymers varies. Because of this, glucoamylase may be the most important enzyme for digesting complex carbohydrates. Eventually, this finding may help manufacturers improve baby foods and formulas.

For now, the best way to overcome stunted infant growth is to increase the



Richard Schanler (left) and Cutberto Garza monitor the feeding of a 7-week-old premature infant in the intensive care unit of Texas Children's Hospital in Houston. (0986X1109-23a)

infant's dietary intake by feeding a standard infant formula and table foods as soon as possible after a lengthy illness.

Parents, of course, should follow their doctor's advice, says pediatrician Kathleen J. Motil. "But it may be necessary to boost a formula's calorie content by supplementing it with corn syrup, corn oil, or commercially prepared glucose polymers."—By **Sam Shaffer**, ARS (retired). **E. Roseland Klein**, Scientific Editor for the Children's Nutrition Research Center, contributed to this article.

*Scientists mentioned in this article are at the USDA-ARS Children's Nutrition Research Center, 6608 Fannin St., Houston, TX 77030. ■*

# TECHNOLOGY

## <sup>245</sup> An Apple a Day All the Year Round <sup>4</sup>



In a controlled atmosphere commercial fruit storage facility at Wenatchee, WA, where ARS testing takes place, plant physiologist Kenneth Olsen checks apples for low-oxygen and low-temperature injury. Olsen wears breathing mask and air tank in the 1-percent oxygen atmosphere. (0187X011-8A)

You can buy crisp, juicy apples and pears from the Pacific Northwest any time of the year—and almost anywhere in the United States—mostly because of advances in storing these fruits made over the last 30 years.

And because today's customers want stored apples to taste as good as those that have been freshly picked, the newest U.S. Department of Agriculture research in Wenatchee, WA, has turned to flavor.

Horticulturist Stephen R. Drake with USDA's Agricultural Research Service and biochemist John K. Fellman of the Washington State Tree Fruit Commission are monitoring storage rooms to determine the special flavor and odor essences given off by ripening apples. Their goal: to prevent flavor losses during the long months in storage or somehow remedy them when apples are taken out of storage and sent to the supermarket.

Marketing of both Red Delicious and Golden Delicious apples has been revolutionized in recent years by storage in special cold rooms that are low in oxygen and high in carbon dioxide. This technique is known in the industry as controlled atmosphere (or CA) storage.

"This approach is clearly the culmination of 20th-century cold-storage research," says plant physiologist Max W. Williams, research leader at the ARS Tree Fruit Research Laboratory in Wenatchee. "Before, growers grew only what could be sold within 4 to 5 months of harvest. There was no point in growing any more apples because in regular cold storage, they would soon get mushy or rot. Now we can keep fruit in good condition up to a full year."

How do controlled atmosphere and regular storage differ? In both, the temperature is kept at just above freezing. But controlled atmosphere has two other critical requirements.

The first is that fruit be picked after it has matured but just before it's had a chance to fully ripen. Apple maturity tests conducted regularly by the Washington State apple industry help growers decide the best time to harvest their fruit and get it into storage.

Each week, lab technicians examine fruit from orchards throughout the

Doug Wilson

state for five key indicators of ripeness: firmness, sugar-to-acid ratio, soluble solids (the ratio of water to fiber), starch content, and ethylene (a natural hormone considered to be the agent that triggers the ripening process).

The second requirement for long-term storage is that the oxygen and carbon dioxide levels be strictly regulated. Storage rooms typically contain 5 to 20 times less oxygen and 8 times more carbon dioxide than the air we breathe. It's this combination of low oxygen and high carbon dioxide that slows down the rate at which fruit ripens and thus allows fruit to be stored so long. "But, you have to be careful," says Williams. "In our early experiments, we found that too much carbon dioxide caused brown patches in fruit and too little oxygen 'suffocated' fruit and caused it to go soft and die."

The cold temperatures and thin air mean that workers have to wear breathing gear when they're in the concrete-walled rooms. They also work in pairs in case one of them should have an accident and need help.

Ordinary cold storage rooms cost about 2/3 that of controlled atmosphere to build and run. But because controlled-atmosphere storage extends the life of apples 5 or 6 months, the additional cost is more than offset, Williams says.

According to Williams, recent research advances have meant growers could expand their orchards and produce more crops to be stored year-round. New storage techniques have almost doubled the amount of apples Pacific Northwest growers can sell: Washington State apple production has increased from 30 million boxes to 55 million boxes over the last 20 years and could increase to 70 million boxes by 1990.

Each year, part of the crop is sold right after picking—without special storage—in September, October, and November. But 50 to 70 percent of the crop is held in storage, to be sold gradually over the coming 8 to 10 months, until the next year's crop is ready for harvest.

Controlled atmosphere storage began in England in 1929 and was introduced to the United States in 1933. Around 1957, ARS researchers in Wenatchee began to play a major role in adapting this technology to meet the



Doug Wilson

At the Wells and Wade Fruit Company of Wenatchee, Olsen and refrigeration engineer Dick Simmons discuss controlled atmosphere storage. The two buildings in the background have a total controlled atmosphere storage capacity of almost a half million bushels of apples. (0187X009-10A)

specific needs of Pacific Northwest growers.

"There are dozens of varieties of apples, and every one takes a different mixture of gases," says Williams. "We worked out the best ones for storing Red Delicious and Golden Delicious, the two major varieties of apples grown in the Pacific Northwest. Now we want to bring out the best flavor."

Williams singles out plant physiologist Kenneth L. Olsen, a former colleague at the Wenatchee lab who retired in 1986, as the scientist who played the key role in developing controlled-atmosphere storage in the Pacific Northwest. "It was he who found the right recipe for treating Golden Delicious apples and showed that apples must go into storage within 7 to 10 days after harvest. His methods are now widely used by apple producers," says Williams.

Olsen's other accomplishments read like a short history of high-tech apple storage:

- He developed specific techniques for extending the storage life not only of Golden and Red Delicious apples and d'Anjou pears, but also of cherries and nectarines.

- He recommended that certain

varieties of apples and pears be harvested even earlier than the already early harvest controlled atmosphere requires and thus dramatically increased their storage life.

- He observed and evaluated the effects of long-term storage on fruits and vegetables.

- He showed that controlled-atmosphere storage of d'Anjou pears requires conditions similar to those for Delicious apples and that moderate levels of carbon dioxide prevent a storage condition of pears known as "pithy brown core."

- In cooperation with ARS transportation engineers, Olsen experimented with different arrangements for stacking fiberboard boxes on pallets for maximum air movement and optimum cooling of fruit.

George Ing, Chairman of the Washington State Tree Fruit Research Commission, says, "Olsen contributed immensely to our knowledge about the storage of apples."—By Howard Sherman, ARS.

Max W. Williams, Stephen R. Drake, and John K. Fellman are at the USDA-ARS Tree Fruit Research Laboratory, 1104 North Western Avenue, Wenatchee, WA 98801. ■

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## PATENTS

### Fungus To Control Fusarium Wilt

See "Biological Defense for Many U.S. Crops," page 6. *Patent No. 4,489,161*, "Strain of *Trichoderma viride* To Control *Fusarium Wilt*"; *Patent No. 4,246,258*, "Biological Control System"; and *Patent Application Serial No. 650,739*, "Method for Screening Bacteria and Application Thereof for Field Control of *Pythium spp.* on Small Grain Crops." ■

### Instant Timed-Release Capsules

A new way to control release of insecticides, herbicides, and other biologically active agents has been developed by an ARS scientist at New Orleans, LA.

As one example using this method, a herbicide is mixed with a solution of algin (derived from seaweed). Droplets of the mixture are then allowed to fall into a gelling solution. The slow-release gel beads form almost immediately on contact with the solution. The rate at which the herbicide is ultimately released can be slowed even further by drying the beads in small pellets.

The biodegradable beads formed in this process can be made to float for

use on water surfaces by mixing air into the alginate formulation before the beading process. The new process can also be made continuous by removing the beads as they form and maintaining the strength of the gelling solution.

For technical information, contact William J. Connick, Jr., USDA-ARS Southern Research Center, P.O. Box 19687, New Orleans, LA 70179. *Patent No. 4,401,456*, "Controlled Release of Bioactive Materials Using Alginate Gel Beads." ■

### Red Blood Cells as Timed-Release Capsules

Drugs to protect livestock from bloodsucking insects and other parasites now can be encapsulated in red blood cells.

Red blood cells are converted into timed-release capsules by taking blood from an animal and isolating the red cells. Then the cells are placed in a dialysis bag where they are made to swell until microscopic pores develop within their membranes. Once these pores are open, the cells are removed from dialysis and mixed with whatever drug is desired for the animal. Later, when the salt concentration of the cells is restored, they shrink to normal size. The pores close, trapping the drug inside.

For technical information, contact John R. DeLoach, USDA-ARS Veterinary Toxicology and Entomology Laboratory, P.O. Drawer GE, College Station, TX 77841. *Patent No. 4,389,209*, "Method of Providing Systemic Protection From Bloodsucking Insects and Other Parasites Using Encapsulated Additives in Resealed Erythrocytes." ■

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If you are interested in applying for a license on a patent or receiving a catalog of USDA patents, write to the Coordinator.

Copies of existing patents may be purchased from the Commissioner of Patents and Trademark Office, Washington, DC 20231. Copies of pending patents may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161. ■